SPONTANEOUS ALTERATIONS IN CHROMOSOME SIZE AND FORM IN ZEA MAYS

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Spontaneous aberrations in maize leading to changes in size and form of the chromosomes have not been investigated from the point of view of determining, systematically, the frequency and positions of breakages and reunions of broken ends of the chromosomes of the complement, as has been done in Tradescantia (Giles, 1940), in Allium (Nichols, 1941) and in other forms (Darlington and Upcott, 1941). Nevertheless, through studies of various problems not directed toward this goal, much has been learned of the process underlying the origin of changes in size and form of the chromosomes of maize which are not conditioned by the usual methods of inducing aberrations, such as X-radiation, ultraviolet radiation, high temperatures and aging.

In the early cytological studies of maize, it became clear that spontaneous aberrations were occurring to give rise to various types of altered chromosomes. In many cases, the time of occurrence or the conditions which gave rise to the alteration were not known. These aberrations were first observed in various plants of particular strains which were under cytogenetic investigation. These aberrations included reciprocal translocations, inversions, deficiencies, ring-chromosomes, a duplication, fragments, and a secondary trisome. Through further studies, it became apparent that chromosome modifications were occurring in individual plants under investigation. A single plant of a culture may show one of the various types of aberrations mentioned above. Although, in some cases, it could not be determined whether all of the cells of the plant possessed the aberration, in other cases it was determined that the plant was sectorial for the modification. In these latter cases, it was obvious that the modification occurred during the development of the individual plant. The factors responsible for these spontaneous aberrations were not apparent in any of these cases. However, there are types of chromosomal aberrations which are induced by known factors or are correlated with known conditions. These will be considered under appropriate headings in the following discussion.

Spontaneous Chromosome Aberrations under Genic Control

There are two well investigated cases which indicate that the rate of spontaneous chromosome aberration in maize may be controlled by the genic composition of the nucleus. The first case is strikingly illustrated by the *sticky* gene studied by Beadle (1932, 1937). *Sticky* is a recessive mutant located in chromosome 4 which causes a tremendous increase in the rate of spontaneous chromosome aberration in all types of tissues. At the first meiotic anaphase in homozygous plants, the chromosomes appear adhered to one another. This sticking together of the chromosomes of the complement suggested the designation *sticky* for this mutant. As a consequence of this sticking, many of the chromosomes are ruptured during the meiotic anaphase. In the mitotic divisions, numerous types of chromosomal aberrations were observed in plants homozygous for the sticky mutant. The continued production of spontaneous aberrations during development causes plants homozygous for *sticky* to be stunted in growth and to possess numerous streaks of tissues with altered phenotypes. The endosperm tissues are likewise a mosaic of various types of aberrant cells. The extremely high rate of spontaneous chromosome alterations, both in the sporophytic and endosperm tissues, undoubtedly is the cause of the observed phenotypic alterations of the cells and tissues, for various grades of chromosomal unbalance must be present in these cells. It is likewise of particular interest to note that the *sticky* mutant is responsible for a marked increase in the rate of spontaneous mutation.

The second case of spontaneous chromosome alteration under genic control has been studied by Jones (1937, 1940). In some of his strains of maize, the endosperm tissues give unmistakable evidence of a high rate of spontaneous chromosome aberration. Unlike the *sticky* mutant, the high rate of chromosome aberration appears to be confined to the endosperm tissues alone. The genetic evidence indicates that reciprocal translocations are occurring between non-homologous chromosomes and that chromosomes with unstable broken ends are likewise produced. Other types of chromosomal aberrations could not be detected genetically. Cytological observations of the endosperm tissues of these plants have produced direct evidence of a high rate of spontaneous chromosome aberration (Clark and Copeland, 1940).

It is needless to say that any systematic study of spontaneous chromosome aberration in maize must be considered with reference to the genic composition of the plants under investigation.

Chromosome Alterations Induced by Crossing over Between Homologous Segments of Chromosomes

The normal process of crossing-over may be responsible for the production of chromosomes with

altered sizes and forms. When individual plants are heterozygous for an inversion, a duplication or some rearrangement in the linear organization of the chromosome, predictable types of chromosomal alterations may follow crossing-over between homologous segments of chromosomes. Although structural heterozygosity greatly increases the rate of production of altered chromosomes following crossing-over, it will be shown that chromosome aberrations likewise may be induced within a normal complement following the regular process of crossing-over. Several examples illustrating the part that crossing-over plays in the production of chromosomal aberrations will be considered.

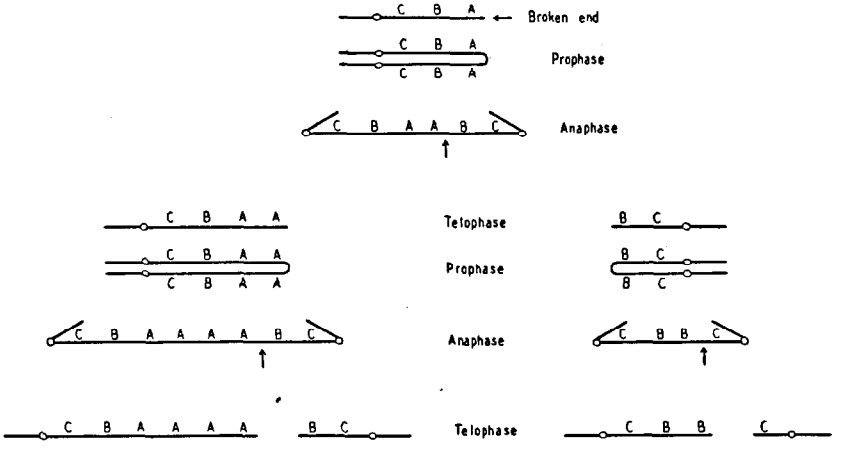


FIG. 1. Diagrammatic representation of the method by which a chromosome with a broken end gives rise to chromosomes with altered sizes and constitutions. The diagram at the top of the figure represents a chromosome with a broken end. The centromere is represented by the clear oval. The organization of the arm with the broken end is represented by A, B and C, A being adjacent to the broken end. Following reduplication of the chromosome, the two sister chromatids are fused at the position of previous breakage (Prophase, second diagram from top). The centromeres pass to opposite poles in the succeeding anaphase. This produces a bridge configuration (Anaphase, third diagram from top). If breakage of this bridge configuration occurs at the position of the arrow, a broken chromosome will enter each telophase nucleus (Telophase, right and left, fourth diagram from top). The broken chromosome to the left possesses a duplicated segment, that to the right is deficient for a terminal segment. Continuation of this breakage-fusion-bridge cycle in succeeding nuclear divisions may result in the production of chromosomes with various duplications, deficiencies or duplications plus deficiencies as illustrated in the diagrams below each of these telophase chromosomes. (From McClintock 1941a, through the courtesy of *Genetics*.)

Plants heterozygous for an inversion which does not include the centromere will give rise to altered chromosomes following a crossover within the inverted segment. It is well known that this results in the production of a dicentric chromatid and an acentric fragment. Passage of the two centromeres of the dicentric chromatid toward opposite poles in the meiotic anaphase spindle produces a chromatin bridge configuration. Rupture of this bridge occurs either before or following the formation of the cell plate. The position of rupture varies. It may be adjacent to one centromere or at any position between the two centromeres. In all cases, a ruptured chromatid possesses a deficiency because the dicentric chromatid itself is deficient for a segment carried by the acentric fragment. If the position of breakage in the bridge configuration is non-median, the broken chromatid entering one nucleus will be more deficient than the broken chromatid entering the sister nucleus. The latter broken chromatid will possess a duplication besides a deficiency. Several such inversions have been investigated in maize (McClintock 1933, 1938b). In several cases, a deficient broken chromosome, presumably derived from crossing over within an inverted segment, has been recovered in the following plant generation.

The breakage of a chromosome at a meiotic anaphase is the starting point in the production of chromosomes with various modifications of size and chromatin constitution. The direct cause of these modifications is related to the subsequent behavior of the broken end. When a chromatid is broken at a meiotic anaphase, fusions occur at the position of breakage between the two sister halves of this broken chromatid (Prophase, second line, fig. 1). As the centromeres of the terminally fused sister chromatids pass to opposite poles in the following mitotic anaphase, a chromatin bridge configuration is produced (Anaphase, third line, fig. 1). As the centromeres approach the poles of the spindle figure, tension is exerted on this bridge causing it to rupture. The rupture may occur at any position between the two centromeres. Following a non-median rupture of the bridge configuration (arrow, upper Anaphase, fig. 1) the broken chromosome entering each sister telophase nucleus will differ in chromatin constitution (upper Telophase, right and left, fig. 1). The behavior of the broken end in each daughter nucleus is similar to its behavior in the previous mother nucleus. Following reduplication of

the chromosomes, fusion occurs between the two sister chromatids at the position of the last breakage and a bridge configuration is produced in the succeeding anaphase which is followed by rupture and the inclusion of a chromosome with a broken end in each sister telophase nucleus. If this process continued, each succeeding mitosis would possess an anaphase bridge configuration because each preceding telophase nucleus had received a chromosome with a broken end. The continuation of this breakage-fusion-bridge cycle should produce chromosomes with various deficiencies, duplications and reduplications of segments following non-median rupture of the bridge configurations in successive anaphases. This subsequent behavior is illustrated in Figure 1. Thus, the production of a dicentric chromatid following crossing-over at a meiotic prophase may initiate a breakage-fusion-bridge cycle. It has been demonstrated that this cycle will continue in all subsequent gametophytic and endosperm mitoses following its origin at a meiotic anaphase (McClintock 1939, 1941a). However, this cycle will cease whenever such a broken chromosome is delivered to the zygote. The broken end heals. This healing is permanent for no further fusions and breakages will occur in the sporophytic mitoses or in any tissues of succeeding plant generations. Because this cycle occurs in the gametophytic divisions (two in the male, three in the female) preceding the formation of the zygote, a wide range of newly organized chromosomes with stable broken ends could be recovered in the sporophytic tissues. These could possess deficiencies of various lengths, duplications of various lengths, deficiencies plus duplications or simple or multiple duplicated segments if the original broken chromosome possessed at least a complete complement of genes of the chromosome.

Although plants heterozygous for an inversion which does not include the centromere produce chromosomes with broken ends at meiotic anaphases following crossing-over within the inverted segment, these inversions usually may not be used to recover chromosomes with modified constitutions because all of these broken chromatids are deficient for a segment of chromatin. In most cases, the deficiency in the genomic complement of the spore receiving a broken chromatid is sufficient to hinder the functioning of the gametophyte arising from it. Consequently, other structural modifications have been used which will produce at meiosis a chromatid with a broken end but with no deficiency of genes of this chromosome. A spore nucleus receiving such a broken chromosome has no deficiency in its genomic complement. A functional gametophyte could be produced from such a spore. Two alterations in the structural composition of chromosome 9 in maize have been used for this study. One is a moderately complex rearrangement of segments composing the chromosome (fig. 2: a, normal chromosome 9; b, rearranged chromosome 9). In the second case, the chromosome 9 possessed a duplication of the short arm as shown in Figure 3. In each of these two cases, a dicentric chromatid is produced following a crossover between the normal chromosome 9 and the modified chromosome 9 (d, fig. 2; c, fig. 3). If breakage of the dicentric chromatid occurred at or to the left of the arrow in the

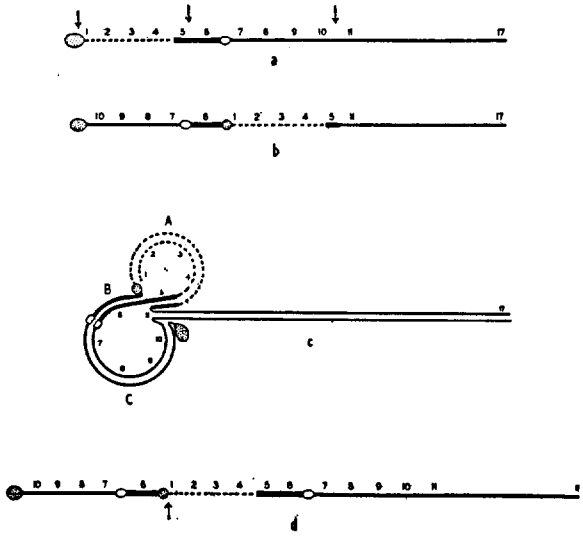


FIG. 2. Diagrammatic representation of a rearrangement in chromosome 9 which may lead to the production of a broken chromosome with at least a full complement of genes for this chromosome. *a*, a normal chromosome 9. The centromere is represented by the clear oval. The short arm terminates in a large knob. The linear organization of the chromosome is represented by the dash line, the heavy line and the lighter line aided by the numerals. By means of X-rays, the chromosome was broken at the positions of the arrows. Union of broken ends gave rise to the rearranged chromosome 9 as shown in *b*. The synaptic association of this chromosome and a normal chromosome 9 (with no terminal knob) is shown in *c*. A crossover in region *A* will produce the dicentric chromosome shown in *d*. This dicentric chromosome possesses a full complement of genes of chromosome 9 from the arrow to the right end of the chromosome. A bridge configuration results in anaphase I. If a break occurs at or to the *left* of the arrow, the broken chromatid to the *right* will possess at least a full complement of genes of this chromosome. (From McClintock 1941a.)

diagrams, the broken chromatid to the *right* would possess at least a complete set of genes for this chromosome. The breakage-fusion-bridge cycle which occurs in the subsequent gametophytic divisions could produce chromosomes with various constitutions by the method illustrated in Figure 1. Thus, chromosomes 9 with the various modifications described above could be delivered to the zygote. A wide range of structurally modified chromosomes 9 have been recovered in the progeny of inviduals heterozygous for these two modifications (McClintock, 1941a).

It may be seen that the breakage-fusion-bridge cycle is a particularly favorable means of obtaining

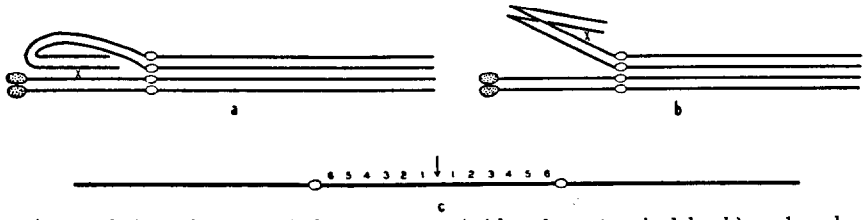
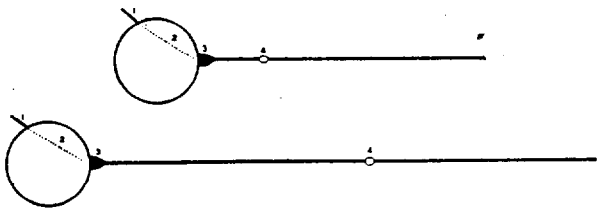


FIG. 3. The synaptic association of a normal chromosome 9 (with a large terminal knob) and a chromosome 9 with a duplication of the short arm in the inverted order (no knob present). The clear oval represents the centromere. A crossover as indicated, following the association in *a*, will produce the dicentric chromosome shown in *c*. Likewise, a crossover as indicated, following the association shown in *b*, will produce the dicentric chromosome shown in *c*. This dicentric chromosome is equivalent to two chromosomes 9 fused at the ends of their short arms. This dicentric chromosome produces a bridge configuration at a meiotic anaphase. If the break occurs at or to the *left* of the arrow, the broken chromatid to the *right* will possess at least a full complement of genes of this chromosome. (From McClintock 1941a, through the courtesy of *Genetics*.)

chromosomes with altered sizes and chromatin constitutions. It is not understood why this cycle is confined to the gametophytic and endosperm tissues in the generation immediately following the meiotic origin of the broken end nor why it ceases in the sporophytic tissues and never reappears. The recovered broken chromosome is as permanent in its morphology as any normal chromosome of the complement. The behavior of a chromosome initially broken in the sporophytic tissues is not the same and will be discussed later.

In several dissimilar cases the normal process of crossing-over has been held responsible for alterations in the structural composition of the chromosome. Two of these will be mentioned (McClintock, unpublished). The first case involves the nucleolus chromosome. The appearance of the nucleolus chromosome at prophase is diagrammed in Figure 4. The nucleolus organizer, a deep staining body adjacent to the nucleolus, is responsible for the organization of the nucleolus at telophase (McClintock, 1934). During this process, the segment of chromatin from the organizer to the end of the short arm (the satellite) is removed from the main body of the chromosome by growth of the nucleolus. Although removed some distance from the organizer, it is attached to it by a thread running through the nucleolus substance. If at a meiotic prophase, a chiasma forms between the centromere and the nucleolus organizer and if terminalization of this chiasma proceeds toward the end of the arm of the chromosome, will the terminalizing chiasma stop at the organizer or will it pass through the nucleolus substance to reach the end of the arm? It apparently cannot pass through the nucleolus. The terminalization process either stops at the nucleolus organizer, or, if the force is great enough, the chromatids involved are ruptured at the position of attachment of the organizer to the nucleolus. Following this rupture, fusion occurs at the position of breakage between the nucleolus organizers of the two chromatids involved. Consequently, a dicentric chromosome is formed which results in a bridge configuration at a meiotic anaphase. It will be noted that the segment between the centromere and the



FIGS. 4 AND 5

FIG. 4 (above): Diagrammatic illustration of the normal nucleolus chromosome in maize. The large circle represents the nucleolus. The small, clear oval (4) represents the centromere. The large, deep-staining body (3) attached to the nucleolus represents the nucleolus organizer. The satellite is represented by 1. Because of the growth of the nucleolus at telophase, the satellite is removed from the nucleolus organizer but remains attached to it by a thread (2) which is in or on the nucleolus itself. This condition is maintained from telophase to the following late prophase. If, in a normal plant, a chiasma forms between the nucleolus organizer and the centromere (between 3 and 4) and if terminalization of this chiasma proceeds toward the end of the arm, obstruction occurs when the chiasma reaches the nucleolus. The nucleolus organizers may be ripped from the nucleolus resulting in breakage of the chromatids at this position. Fusion 2-by-2 then occurs between the broken chromatids at the position of rupture. This produces a dicentric chromatid composed of two chromosomes 6 fused at the distal part of their nucleolus organizers. Breakage of this dicentric chromosome at various positions between the centromeres during the following meiotic anaphases produces chromosomes with various modifications in size and chromatin content.

FIG. 5 (below): The nucleolus chromosome in a plant homozygous for a translocation between chromosome 6 and chromosome 5. The description of this chromosome is similar to that given in the legend of Figure 5. The translocation occurred adjacent to the centromere on the short arm of a normal chromosome 6 and toward the end of the long arm of a normal chromosome 5. The centromere of the resulting nucleolus chromosome is located a considerable distance from the nucleolus organizer. Chiasma formation between region 3 (the nucleolus organizer) and region 4 (the centromere) is very frequent in plants homozygous for this translocation. Consequently, the chromatids are frequently ruptured at the attachment of the nucleolus organizer to the nucleolus during terminalization of these chiasmata. Dicentric chromatids are produced following 2-by-2 fusions of ruptured nucleolus organizers.

nucleolus organizer where an effective chiasma could be formed is relatively short in the normal nucleolus chromosome. Relatively few bridge configurations following this process appear at meiotic anaphases. The true nature of these bridge configurations was clearly revealed during a study of meiosis in plants homozygous for a translocation which placed the centromere at a considerable distance from the nucleolus organizer. This translocation chromosome is diagrammed in Figure 5. Chiasma formation is very frequent in the long segment between the centromere and the nucleolus organizer. Many bridge configurations arising from fusions of ruptured nucleolus organizers were observed at meiotic anaphases in these plants. Rupture of the anaphase bridge configurations at various positions between the two centromeres gives rise to chromosomes with variously modified constitutions. As expected, they include various degrees of duplication or deficiency. Their constitutions may be observed readily in the prophases of the following spore divisions. Thus, if chiasmata are the result of crossing-over, as the combined evidence suggests, the normal process of crossing-over may be a factor in the origin of modified chromosomes even when no structural rearrangements are present.

The second case of alteration in the constitution of chromosomes for which crossing-over is held responsible is again related to chiasmata. In several cultures of maize, it has been observed that both the terminal and the interstitial chiasmata in all of the chromosomes of the complement are released or unraveled only with considerable difficulty at the first meiotic anaphase. Consequently, as the disjoining centromeres of the bivalent chromosomes pass toward opposite poles, the chromatin between the centromeres and the chiasma is drawn out into a very fine thread. Frequently the tension becomes great enough to rupture these threads before the chiasma has unraveled and a broken, deficient chromosome enters the telophase nucleus.

From the evidence reviewed, it may be seen that the normal process of crossing-over is a means by which chromosomes with altered constitutions are produced.

Alterations in Size, Form and Constitution of Chromosomes Following Non-Homologous or Illegitimate Crossing-Over

Synaptic associations involving non-homologous parts of chromosomes are regularly present in maize when the chromosome complement is heterozygous for some structural rearrangement or when an unbalanced chromosome complement is present. The nature of this association has been extensively investigated (Burnham, 1932; McClintock, 1932, 1933). The synaptic behavior of the univalent chromosome in monosomic plants or of the extra chromosome in trisomic plants may be used as an example. The non-homologous synaptic associations of the univalent at the meiotic prophase are variable but one of the most frequent types of associations is diagrammed in *a* Figure 6. This 2-by-2 association is completely non-homologous. Breakage of the chromatin threads and reunions of the broken ends following an illegitimate crossover at $x - x'$ could give rise to a chromosome with an inverted segment or to a deficient rod-shaped chromosome and an acentric ring-shaped chromosome depending upon whether the reunion of the broken ends was diagonal

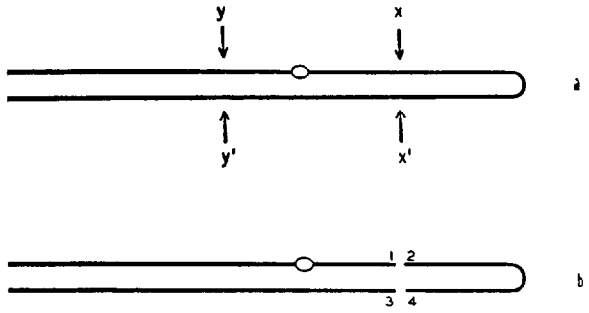


Fig. 6. Diagram representing the non-homologous synaptic association of a univalent chromosome. The clear oval represents the centromere. The chromosome is folded upon itself at the mid-region. The association is completely non-homologous. If an illegitimate crossover occurs at x-x' there are two possible consequences depending upon the resulting 2-by-2 fusions of broken ends. These broken ends are designated 1, 2, 3 and 4 in *b*. The fusion of alternate broken ends, 1 with 4 and 2 with 3, will result in a chromosome with an inverted segment. Fusions of opposite broken ends, 1 with 3 and 2 with 4, will give rise to a deficient rod-shaped chromosome and an acentric ring-shaped chromosome. If the breaks occurred at y-y' in *a*, fusions of alternate broken ends will give rise to a chromosome with an inversion. Fusions of opposite broken ends will give rise to a deficient ring-shaped chromosome possessing the centromere and a deficient, acentric rod-shaped chromosome.

or opposite, respectively (*b*, fig. 6). If the illegitimate crossover occurred at $y - y'$, fusions of diagonal broken ends would produce an inversion while opposite fusions of broken ends would produce a deficient rod-shaped chromosome without a centromere and a ring-shaped chromosome possessing the centromere. With respect to opposite fusions, the deficient rod chromosome could be recovered from the $x - x'$ "crossover" whereas a deficient ring-shaped chromosome could be recovered from the $y - y'$ "crossover." It is interesting to note that just these types of modified chromosomes have appeared in the progeny of trisomic plants. Although no effort has been made to obtain the frequency of these events, the interpretation of their origin as a consequence of illegitimate crossing-over between synapsed non-homologous segments of chromosomes is strengthened by the types of individuals which are occasionally produced in the progeny of plants heterozygous for reciprocal translocations. Heterozygous translocations frequently exhibit extensive non-homologous associations at meiotic prophases. Secondary translocations involving the same

two chromosomes have been recovered from such heterozygous plants. From knowledge of the types of non-homologous synaptic configurations which were known to be present in the parent plant, these secondary translocations may readily be derived on the hypothesis of illegitimate crossing-over.

Haploid plants are characterized by very extensive non-homologous associations. This process may be initiated in some parts of the complement by homologous attractions of unidentified duplicated segments but much of the observed 2-by-2 synaptic association is definitely non-homologous. At the first meiotic anaphase, several of the chromosomes may be associated, 2-by-2, by what appears to be a chiasma. Fragments of various sizes may likewise be present. Both the chromosome associations and the fragments may well arise as the consequence of illegitimate crossing-over between associated non-homologous segments of chromosomes although legitimate crossing-over between homologously associated duplicated segments has not been excluded. As yet, we do not know whether such duplicated segments are present in the complement of maize. A more detailed study of the types of chromosome associations and aberrations at meiosis in the haploid plants or a study of the chromosome complements of the progeny of haploid plants could distinguish between legitimate and illegitimate crossing-over. The legitimate crossovers would be expected to give the same chromosomal rearrangement on a number of independent occasions. On the other hand, illegitimate crossing-over following non-homologous associations would not be expected to occur at the same position on a number of independent occasions. The progeny of haploid plants has not been extensively investigated but it is expected that chromosomes with altered constitutions would appear.

New Types of Chromosomes Arising from the Aberrant Behavior of a Telocentric Chromosome

It has been suspected for some time, on good observational evidence, that true telocentric chromosomes—that is, chromosomes with strictly terminal centromeres—normally are not present in the chromosome complements of organisms. From these observations, one could conclude that some aberrant behavior of telocentric chromosomes must result in their elimination from the complement or that they become modified in such a way that a true telocentric condition no longer exists. In a recent study by Rhoades (1940) the behavior of a strictly telocentric chromosome has been investigated. The suspicion, based on deductive evidence, that telocentric chromosomes are unstable has been confirmed by this investigation. This telocentric chromosome investigated by Rhoades was discovered in a single plant in the progeny of an individual trisomic for chromosome 5. It was composed of a complete short arm of chromosome 5 with the proximal end terminating in the centromere. No chromatin extended beyond the centromere. Both genetic and cytological evidence indicates that the mitotic behavior of the telocentric chromosome is normal in the majority of mitoses. In some mitoses, however, its behavior must be aberrant. Although these aberrant mitoses have not been observed directly, they may be inferred from the genetic behavior and the types of altered chromosomes which are derived from the telocentric chromosome. Plants were obtained with two normal chromosomes 5, each carrying the recessive mutant *bm* (brown mid-rib, located in the short-arm adjacent to the centromere), and a telocentric chromosome carrying the dominant allele, *Bm*. Variegation for *Bm* and *bm* appeared in some of these plants. It was concluded that this variegation was related to aberrant behavior of the telocentric chromosome which either eliminated the telocentric chromosome from some nuclei or eliminated the *Bm* locus from this chromosome. In several cases, a *bm* sector extended into the tassel. This allowed a cytological determination to be made of the chromosome complement of such a sector. In one such case, the observations showed that the telocentric chromosome had been completely eliminated from the nuclei of the sector. In four other such cases, the original telocentric chromosome had undergone considerable modification. In two of these cases, the telocentric chromosome had been modified and reduced to a small fragment with a *subterminal* centromere. In a third case, a telocentric chromosome was present but its size was only one-half that of the parental telocentric chromosome. In the fourth case, a minute fragment was present composed of only two or three chromomeres and a terminal centromere. Although the observational evidence is insufficient to indicate the methods of origin of these modifications of the original telocentric chromosome, it does indicate that telocentric chromosomes are unstable. They may be eliminated totally from the nuclei or they may produce variants with decidedly altered constitutions.

One recurring type of modification suggests the nature of one type of instability of the telocentric chromosome. When a plant containing this telocentric chromosome in addition to the normal complement is crossed by or onto normal plants, three types of plants are expected in the progeny. These are (1) normal diploids, (2) plants trisomic for chromosome 5 and (3) plants carrying the telocentric chromosome in addition to the normal chromosome complement. Such plants appear in their expected proportions when the female parent carried the telocentric chromosome. Pollen grains carrying either an extra chromosome 5 or the telocentric chromosome rarely function in competition with grains carrying a normal chromosome complement. However, an unexpected type of plant appeared in approximately the same relative proportions in the progeny of these reciprocal crosses. These plants possessed an extra chromosome. This extra chro-

mosome was composed of two short arms of chromosome 5 joined by a single median centromere—a true isochromosome. It is known that pollen grains carrying such an isochromosome in addition to the normal complement do not function in competition with normal grains. However, in the case mentioned, sperm nuclei carrying isochromosomes were delivered to egg nuclei.

It is necessary to explain first the origin of this isochromosome and secondly, how a pollen grain may deliver such a chromosome to the egg nucleus. If one assumed that normal reduplication of the chromatin of the telocentric chromosome occurred in some of the mitoses in the plant carrying the telocentric chromosome, which was accompanied by some form of misdivision of the centromere of this chromosome either in the prophase or in the subsequent spindle figure, an isochromosome in addition to the normal complement could enter one telophase nucleus. Under these circumstances, only the normal chromosome complement could enter the sister telophase nucleus. If this occurred during the division of the microspore nucleus, a generative nucleus carrying an isochromosome and a tube nucleus carrying only the normal complement could be produced. It is assumed that the functioning of a pollen grain is controlled by the constitution of its tube nucleus. Thus, a pollen grain could deliver an isochromosome to the egg nucleus if its tube nucleus possessed a normal chromosome complement and its sperm nuclei carried, in addition, an isochromosome. Such misdivision of the centromere of the telocentric chromosome may be one of the factors responsible for the *Bm—bm* variegation mentioned above.

The evidence reviewed indicates that the telocentric condition is another factor leading to the production of a wide range of spontaneous chromosome alterations.

The Relation of Chromosome Form to Constancy of Chromatin Constitution

In the previous sections it was pointed out that permanency of the constitution of a chromosome will not be maintained through successive nuclear cycles if the chromosome possesses an unstable broken end or if it possesses a strictly terminal centromere. Extensive and varied modification in the size and genic content of chromosomes arise from these two conditions. There is a third condition which leads to extensive modification of the composition of a chromosome. If a chromosome has the form of a ring rather than a rod, it does not maintain itself unaltered through successive nuclear cycles. Although its form does not change, its chromatin composition is continuously subject to alteration. The ring chromosomes may become enlarged by duplication and reduplication of segments composing the ring or they may decrease in size by deletions of segments from the ring. Alteration after alteration will occur if the ring *form* of the chromosome is maintained. A plant possessing a ring-shaped chromosome may be a complete mosaic of altered ring-shaped chromosomes. In some cells and tissues the ring chromosome may be deficient for segments of various lengths. In other cells and tissues, the ring chromosome may possess duplications or reduplications of segments. In still other cells and tissues, the ring chromosome may possess both deficient and duplicated segments. The size of the ring chromosome is no indication of its genic content.

Observations of somatic mitoses have indicated the method by which alterations in the chromatin constitution of the ring-shaped chromosome arise (McClintock, 1932b, 1938a, 1941b). It is related to the mitotic cycle. At some mitotic prophases, the two sister halves of a divided ring-shape chromosome form a continuous, double-sized, dicentric ring chromosome instead of two freely separating, monocentric ring chromosomes (Prophase, fig. 7). This condition could arise subsequent to reduplication of the chromonema of the ring chromosome if a somatic crossover occurred between the two sister chromatids, or the reduplication process itself could lead to this condition. At early anaphase, the two centromeres of the double-sized, dicentric ring chromosome move toward opposite poles of the spindle figure (Anaphase, fig. 7). Tension on the chromatin strands between the two centromeres in late anaphase or early telophase causes them to rupture. The position of rupture is variable. Three such possible positions are indicated by the dash lines *a*, *b*, and *c*, respectively (Anaphase, fig. 7). The subsequent behavior of the broken strands is illustrated in the bracketed figures (lower row, fig. 7) for each of these breakages. Segments of the broken double-sized ring chromosome enter each telophase nucleus but the chromatin composition of the segment in the sister telophase nuclei may differ considerably. In each telophase nucleus, fusion occurs between the broken ends of the segment thus reestablishing the ring *form* of the chromosome but not necessarily its original chromatin and genic composition (Telophases, fig. 7). It may be seen that ring chromosomes with duplicated segments of ring chromosomes with deficient segments may be produced by this process. Repetition of this process in a later mitosis could give rise to ring chromosomes with reduplicated or multiple segments of the original ring chromosome, to ring chromosomes with still greater deficiencies or to ring chromosomes with deficiencies plus duplicated segments. The frequency of occurrence of these aberrant mitoses depends on the length of the chromonema of the ring chromosome—the longer the chromonema the more frequent the aberrant mitoses. The aberrant mitotic configurations may occur in twenty percent of all mitoses if the chromonema composing the ring is as long as the longest chromosome of the normal complement. If the ring chromosome is only one-tenth of this size, an aberrant mitosis may occur in only one percent of the mitoses. If the ring chromosome

is only one twenty-fifth of the size of the longest chromosome, an aberrant mitosis may occur in only 0.2 percent of the mitoses. In all other nuclear divisions, the behavior of the ring chromosomes is normal; the two sister halves of the ring chromosome separate freely at anaphase along with the rod chromosomes of the complement.

segments of the dicentric ring chromosome might likewise heal and become stable.

Conclusions

Permanency of chromosome form and constitution through successive nuclear cycles is a basic postulate of genetic theory. This postulate is well

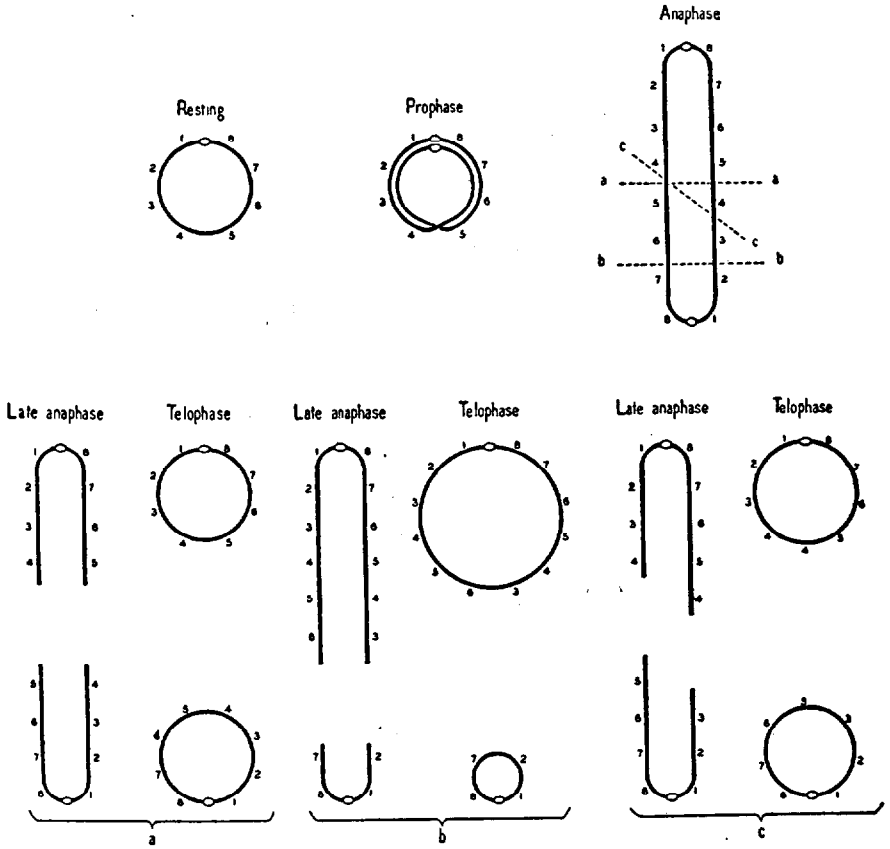


FIG. 7. Diagram illustrating a method by which a ring chromosome becomes altered in chromatin constitution. Upper left: A ring chromosome in a resting nucleus. The clear oval represents the centromere. The individual parts of the ring chromosome are designated by the numerals. Upper middle: A prophase configuration following a "crossover" between the two sister chromatids of the divided ring chromosome. A dicentric, double-sized ring chromosome is produced. Upper right: Appearance of the dicentric ring chromosome in the following anaphase. Breakage of the chromatin strands between the centromeres may occur at any position. Three possible positions *a*, *b* and *c*, respectively, are indicated by the dash lines. The resulting broken strands at late anaphase and the new ring chromosomes formed at telophase by fusions of broken ends of these strands are diagrammed below in the bracketed figures for the breaks *a*, *b* and *c*, respectively. (From McClintock 1941b, through the courtesy of *Genetics*.)

It should be emphasized that fusion of broken ends apparently always follows the breakage of a double-sized, dicentric ring chromosome during an aberrant mitosis in the sporophytic tissues. Although extensively looked for, no cases have been found where the broken ends had failed to unite. Since it has been proved (McClintock, 1941a) that a single broken end which is unstable in the gametophytic tissues may heal and become permanently stable in the following zygote or early sporophyte, it is expected that under certain conditions which at present are not known, the broken ends of the

founded on extensive observational evidence in a wide range of organisms. Knowledge of conditions which will produce changes in this constancy has been of utmost importance in recent years. X-rays, ultraviolet radiation, heat, aging, etc., have been the usual agents producing these desired conditions. It has been known for a long time that other conditions may lead to changes in the form and constitution of chromosomes. When the previous history is not known, the observed changes would naturally fall under the heading of spontaneous aberrations because the conditions responsible for their

occurrence were not apparent. In this discussion, I have attempted to indicate the extent of our knowledge in maize of the conditions which are responsible for such "spontaneous" aberrations. We do know that they may occur (1) under genic control, (2) following legitimate crossing-over, (3) following illegitimate crossing-over, (4) during or following reduplication of the chromonema of the chromosome, (5) as the consequence of the instability of broken ends of chromosomes, (6) during terminalization of chiasmata and (7) as the result of the aberrant behavior of a strictly terminal centromere.

Studies of spontaneous aberrations in maize have contributed and should continue to contribute to our knowledge of the behavior of chromosomes in general. A few such contributions may be summarized. We know from the study of ring-shaped chromosomes that the reduplication process of a chromosome usually occurs along a single plane. The possibility that some sister strand crossover chromatids may be present at meiosis is likewise suggested by these studies for the double-sized dicentric ring chromosomes represent some form of sister chromatid exchange. Although the method of origin of these exchanges is not known, they may be the result of some process which is shared by all chromosomes. If so, the frequency of sister-strand crossover chromatids at meiosis should be directly proportional to the length of the chromosome. However, the process which gives rise to these strands need not be related to the normal process of crossing-over. We are considerably better informed about the stability of broken ends of chromosomes through studies of the types of modified chromosomes which arise following mechanical rupture of chromosomes at meiosis, and following mechanical rupture of dicentric ring-shaped chromosomes at a somatic mitosis. With regard to this, we know that following mechanical rupture of two adjacent chromatids at a late meiotic prophase, fusion may occur between these two chromatids at the position of breakage. However, if these two adjacent chromatids are broken at the first meiotic anaphase, fusions will now occur at the position of breakage between the two sister halves of each of these chromatids. Likewise, if a single chromatid is ruptured at a meiotic anaphase, fusion will occur at the position of breakage between the two sister halves of this chromatid and thus initiate the breakage-fusion-bridge cycle which characterizes the behavior of this chromosome in subsequent gametophytic and endosperm tissues. We know also that fusions may occur between two broken ends of a single chromosome if this single chromosome has suffered mechanical rupture at two points during anaphase of a mitotic division in the sporophytic tissues. From these studies we have determined that a recently broken end of a chromosome is unstable in certain tissues and under certain conditions but may become completely and permanently stable under other conditions. Because of these observations, we know why ring-shaped chromosomes and telocentric chromosomes cannot maintain themselves in nature and thus why they are not frequently encountered. Through the study of the composition of altered chromosomes arising from haploids we may be able to detect the presence of possible duplicated segments in the normal complement of maize which at present are undetected but suspected from genetic evidence. Even the concepts of chiasmata formation and terminalization may be illuminated through the studies of specific chromosome alterations.

It is obvious that the changes in size, form and constitution of chromosomes have made it possible to detect and study some of the processes underlying chromosome behavior in general.

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DISCUSSION

MULLER: What is the rate of apparent "gene mutation" in the sticky stock?

MCCLINTOCK: It is very high, although the homozygotes are very low in fertility. The progeny may also possess chromosomal rearrangements.

GATES: How high is the pollen sterility?

MCCLINTOCK: Very high. Many plants shed no pollen at all.

BOCHE: Would incomplete division be an alternative to the refusion of broken ends?

MCCLINTOCK: We cannot distinguish by our method between incomplete division or fusion following division. All we know is that the two sister chromatids are fused at the position of previous breakage.

MICKEY: Do bridge chromosomes break with greater frequency at one position more often than at any other position?

MCCLINTOCK: Yes, they tend to break at the position of previous fusions.

HUSKINS: If this point were near the middle, the effect could be mechanical.

MCCLINTOCK: Yes, but the break would not be expected to be exactly in the middle, although approaching it.

FANO: Is crossing over involved in the behavior of the ring chromosomes?

MCCLINTOCK: I think somatic crossing over between sister chromatids may be responsible for the double-sized, dicentric ring chromosomes.

MULLER: If a plant is heterozygous for the ring, can such crossing over occur within the ring?

MCCLINTOCK: Yes.

SCHULTZ: Possibly evidence might be obtained from this for sister-strand crossing over at meiosis in maize, which would probably then be more frequent than in Drosophila.

MCCLINTOCK: The behavior of ring chromosomes suggests some form of sister-strand exchange and also suggests that the frequency with which it occurs would depend on the length of the chromonema composing the chromosome. The longer the chromonema, the more frequent the expected occurrence.

NEBEL: Changes in size might also be due to a twist in the plane of reduplication within the ring.

MCCLINTOCK: Yes, it is possible. However, the evidence suggests that this must be infrequent.

MICKEY: Will a gene such as sticky increase the frequency of double sized rings?

MCCLINTOCK: There is no evidence for this in our experiments, but no actual measurements of the frequency in different strains have been made.

SANSOME: Your ring chromosomes came from X-ray treatment, didn't they?

MCCLINTOCK: Not in all cases. Ring chromosomes may arise following X-radiation but also spontaneously.

DELBRÜCK: Do sister-strands heal following breakage of a dicentric ring chromosome?

MCCLINTOCK: I have not found such a case. Such healing would give rise to a rod-shaped fragment. They have been looked for but have not been found. Since broken ends are known to heal under certain as yet unknown circumstances such rod-shaped fragments arising from broken ring chromosomes eventually may be found.

SPARROW: If we assume that the ring chromosome is longitudinally bipartite and that it breaks at anaphase, sister-strand crossing over as shown in the diagram is not necessary to explain double or interlocked rings.

MCCLINTOCK: The diagram represents the simplest interpretation for this presentation.

MULLER and GLASS (simultaneously): Do you get interlocked rings?

MCCLINTOCK: Because of the small size of the mitotic chromosomes in maize, interlocking of two sister ring chromatids would be difficult to detect. At anaphase they would look like a twisted double sized ring; this configuration is frequent but one cannot be sure that it represents interlocked ring chromatids. Interlocked ring chromosomes occur following another type of behavior of ring.

CARLSON: When there is a break followed by refusion between the nucleolus organizer regions, where does the nucleolus form in the daughter cells with reference to the nucleolus organizer?

MCCLINTOCK: Development of the nucleolus takes place at the position of maximum activity of the two fused organizers, which is at the position of fusion of the two organizers.

WARMKE: Concerning the mechanism of bridge formation involving the nucleolus chromosome, if terminalization occurs late, after the nucleolus has disappeared, then does the nucleolus organizer itself inhibit terminalization and thus cause the break?

MCCLINTOCK: Probably not. The breakage is assumed to occur before the nucleolus disappears. The frequency of bridges is low in proportion to the chiasmata frequency or crossing over within this region.

WARMKE: In Datura, crossing over apparently regularly occurs between the centromere and nucleolar organizer, and terminalization takes place with the formation of what have been called humps; chiasmata form without breaks in this case.

MCCLINTOCK: In maize, when the distance between the nucleolus organizer and centromere is short, there are few bridges. As this distance increases, the frequency of the bridges increases. An increase in the distance is obtained from homozygous translocations.